Evidence for marine production of monoterpenes

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Environmental context. Laboratory incubation experiments and shipboard measurements in the Southern Atlantic Ocean have provided the first evidence for marine production of monoterpenes. Nine marine phytoplankton monocultures were investigated using a GC-MS equipped with an enantiomerically-selective column and found to emit monoterpenes including (−)-/(+)-pinene, limonene and *p*-ocimene, all of which were previously thought to be exclusively of terrestrial origin. Maximum levels of 100–200 pptv total monoterpenes were encountered when the ship crossed an active phytoplankton bloom.

Abstract. Laboratory incubation experiments and shipboard measurements on the Southern Atlantic Ocean have provided the first evidence for marine production of monoterpenes. Nine marine phytoplankton monocultures were investigated using a GC-MS equipped with an enantiomerically-selective column and found to emit at rates, expressed as nmol $C_{10}H_{16}$ (monoterpene) g [chlorophyll a]$^{-1}$ day^{-1}, from 0.3 nmol g [chlorophyll a]$^{-1}$ day^{-1} for *Skeletonema costatum* and *Emiliania huxleyi* to 225.9 nmol g [chlorophyll a]$^{-1}$ day^{-1} for *Dunaliella tertiolecta*. Nine monoterpenes were identified in the sample and not in the control, namely: (−)-/(+)-pinene, myrcene, (+)-camphene, (−)-sabinene, (+)-3-carene, (−)-pinene, (−)-limonene and *p*-ocimene. In addition, shipboard measurements of monoterpenes in air were made in January–March 2007, over the South Atlantic Ocean. Monoterpenes were detected in marine air sufficiently far from land as to exclude influence from terrestrial sources. Maximum levels of 100–200 pptv total monoterpenes were encountered when the ship crossed an active phytoplankton bloom, whereas in low chlorophyll regions monoterpenes were mostly below detection limit.

Additional keywords: algae, isoprene, organic trace gases, Southern Atlantic Ocean.

Introduction

Trace organic gas species play several important roles in the atmosphere, influencing ozone photochemistry and aerosol physics (see e.g. ref. [1] and references therein). Many terrestrial sources of such species have been characterised and quantified,[2–4] including anthropogenic emissions associated with the production and use of fossil fuels (e.g. alkanes, alkenes and aromatics), and biogenic emissions from trees and plants (e.g. isoprene and monoterpenes) (see e.g. ref. [4]). In comparison to these extensive terrestrial investigations, relatively little has been done to assess inputs from marine sources. This was in part because of the more limited accessibility, but also because of the perception from earlier oceanic alkane measurements that the ocean is a globally minor source of such species.[5] Recently, however, it has become apparent that the surface ocean can play an important role in the budgets of both organic trace gases and aerosols. For example, the surface ocean has been shown to be a massive reservoir for oxygenated organic species[1,6] and a recent study of marine aerosols at a coastal site in Ireland[7]

showed that the organic fraction contributes significantly (63%) to the sub-micrometer particle mass of aerosols collected over the North Atlantic Ocean during phytoplankton bloom periods. These high-organic-fraction aerosols may be generated directly by bubble bursting at the organic-rich sea surface, or by the emission and subsequent oxidation of biogenic gases that produce semivolatile products and aerosol.

To date very little is known about secondary organic aerosol (SOA) formation from trace organic species released by marine biota. The main exception is dimethyl sulfide (DMS), which is known to be produced biogenically in the ocean[8,9] and yields the inorganic aerosol component sulfate upon complete oxidation in the atmosphere.[10,11] Isoprene, the strongest terrestrial biogenic emission, has also been observed as an oceanic emission[12] and in laboratory based studies of plankton.[13–17] Furthermore, it has also been shown to form aerosols over the tropical rainforest,[18] and more controversially suggested as the cause of cloud droplet radius changes in clouds that form directly over phytoplankton blooms.[19] Surprisingly, several trace gas

compounds that are known to form secondary organic aerosol in the terrestrial environment such as monoterpenes (a diverse group of molecules made up of two isoprene units) (see e.g. refs [20–22]), have never been investigated as emissions from plankton. The natural world is known to use these specific volatile compounds to signal opportunity to insects, pathogens and pollinators alike, or serve as a chemical defence.[23,24] Most of these monoterpenes are chiral, and although in most atmospheric chemistry studies the (+)- and (−)-enantiomers are not resolved, and hence measured together, the individual enantiomers often have markedly different biological properties.[24]

Here we present new data obtained from laboratory cultures of nine phytoplankton species and from shipboard measurements conducted as part of the OOMPH (Organics over the Ocean Modifying Particles in both Hemispheres) summer cruise in the Southern Ocean. Monoterpenes were studied first in the emissions of nine marine phytoplankton species (Coccolithophorid: *Emiliania huxleyi*; Diatoms: *Chaetoceros neogracilis*, *Ch. debilis*, *Phaeodactylum tricornutum*, *Skeletonema costatum* and *Fragilariopsis kerguelensis*; Chlorophyte: *Dunaliella tertiolecta*; cyanobacteria: *Synechococcus* and *Trichodesmium*) and then in the atmosphere over the Southern Atlantic Ocean conducted in January–February 2007 (35°49′S, 20°22′E to 52°17′S, 67°73′W) and 1–25 March 2007 (from 53°10′S, 70°54′W to 20°9′S, 57°29′E). The first leg of the cruise was planned to coincide with the large-scale summer phytoplankton bloom that occurs in the Southern Atlantic Ocean.

Experimental

Incubation experiments

In January–February 2006 several phytoplankton cultures were incubated and their gas phase emissions investigated within laboratories at the Leibniz Institute of Marine Sciences at the University of Kiel (IFM-GEOMAR, Kiel, Germany). Over the course of the experiments, nine phytoplankton species were studied, namely: Coccolithophorids: *Emiliania huxleyi* CCMP 371; Diatoms: *Chaetoceros neogracilis* CCMP 1318, *Chaetoceros debilis* and *Fragilariopsis kerguelensis* both isolated during the Polarstern cruise ANTXXI-3 (EIFEX by L. Hoffmann, IFM-GEOMAR, Kiel and P. Assmy, AWI-Bremerhaven), *Phaeodactylum tricornutum* (Phaeo, originating from the Falkowski laboratory) and *Skeletonema costatum* (AWI-Helgoland-culture collection); Chlorophyte: *Dunaliella tertiolecta* (DUN, originating from the Falkowski laboratory); cyanobacteria: *Synechococcus* RCC40 and *Trichodesmium* IMS101. *Synechococcus* RCC40 was grown in PCR-Tu2-medium[25] and the nitrogen fixing cyanobacterium *Trichodesmium* IMS101 was kept in YBCII medium with no dissolved nitrogen source for growth.[26] All other species were grown in f/2-medium.[27,28]

The cultures chosen during this experiment represent various key species of important biogeochemical provinces. *F. kergulensis* is an endemic species in the Southern Ocean, and *Ch. Debilis*, although also isolated from the Southern Ocean, is known to build worldwide open ocean blooms. *S. costatum* on the other hand is a typical coastal bloom forming species, while *Trichodesmium* is a representative of the nitrogen fixers in the tropical and subtropical regions, an area usually also inhabited by the other cyanobacterium: *Synechococcus*. *E. huxleyi* usually succeeds natural diatom blooms.

For the incubation experiments, duplicate 250-mL glass bottles (Schott, Germany) fitted with Teflon caps (Bola, Germany)

were constructed with three ports to which 1/4-inch (0.635-cm) Teflon tubing could be fixed and through which gas could be flushed for air sampling. A length of Teflon tubing connected a three port Teflon valve at the top of the bottle, to below the liquid surface in the incubation flask so that samples could be periodically drawn out without perturbing the experiment. Within each flask pair, one of the flasks was designated as a control and filled with the growth medium (100 mL) of each algae species, while the second was filled with the investigated algae species in their respective media. Each flask pair was placed in an incubator and the temperature (4°C and 20°C) and PAR maximum intensity (30 or $100\,\mu E\,m^{-2}\,s^{-1}$) were chosen according to the natural habitat of the different cultures, particularly for diatom species characteristic of the Southern Ocean. The light was programmed to follow a 'natural' diurnal light cycle illuminating from 0600 to 1800 hours. To produce a natural light intensity variation, the Light Thermostat was programmed to vary from 0% at 0600 hours, to reach a maximum at noon and thereafter progressively decrease back to 0% at 1800 hours. Synthetic air was flushed continuously through both flasks at identical flow rates ($100\,mL\,min^{-1}$). Air samples were then collected both from the air stream before entering the flasks for air blanks, and also at the exit to determine the net trace gas production of the various species. The difference between the inlet and outlet concentrations was attributed to the biogenic emission (or uptake) of the investigated species if the same difference was not observed in the control flask. The aim of this study was to screen as many phytoplankton species as possible, therefore, there are no replicates for the various species. However, since each species was monitored up to two days, the data presented here are a mean of 16 measurements.

Air samples taken before and after the incubation flasks were collected through stainless steel, two-bed sampling cartridges (Carbograph I/Carbograph II; Markes International, Pontyclun, UK) at a flow rate of $100\,mL\,min^{-1}$. Prior to air sampling, the sampling cartridges were cleaned with the Thermoconditioner TC-020 (Markes International, Pontyclun, UK) by purging with helium 6.0 (99.9999%, Messer-Griesheim, Germany) for 120 min at 350°C and 30 min at 380°C. For storage, the cartridges were sealed with brass caps with PTFE ferrules and put into an airtight metal container (Rotilabo, Carl Roth GmbH & Co, Karlsruhe, Germany). After sampling, the cartridges were stored in a separate airtight metal container for a maximum of 12 h. Shortly before the analysis, the brass caps were exchanged for DiffLok caps (Markes International, Pontyclun, UK).

The average emission rates (taken at an optimal light intensity for each species) of total monoterpenes expressed as nmol total $C_{10}H_{16}$ (monoterpene) g [chlorophyll a]$^{-1}$ day^{-1} emitted by the nine phytoplankton species were calculated using the following formula:

nmol $C_{10}H_{16}$ (monoterpene) g [chlorophyll a]$^{-1}$ day^{-1}

= ([nmol $C_{10}H_{16}$ (phytoplankton)] – [nmol (f/2 medium)])/ chlorophyll a/sampling time

Open ocean experiments

Description of OOMPH cruise

Between January and March 2007, comprehensive shipborne measurements of trace gases and aerosols were made while crossing the South Atlantic Ocean from South Africa to Chile and back as part of the OOMPH (Organics over the

Table 1. Desorption and analysis conditions for laboratory experiments and ship-borne measurements

	Parameters
Thermal desorption	
Primary desorption (cartridges)	Prepurge: 15 min
	Desorption: 10 min, 200°C
	Cold trap: 10°C
Secondary desorption (cold trap)	Desorption: 5 min, 200°C
	Flow path: 140°C
Analysis	
Column	β-cyclodextrin chiral capillary column: Cyclodex-B, 30 m, 0.256 mm I.D., 0.25 μm film (J&W Scientific, Folsom, CA, USA), 40°C, 5 min hold, 1.5°C min^{-1} to 200°C, 5 min hold
Mass spectrometer EI in single ion mode	Potential ionisation: 70 eV
	Source temperature: 230°C

Ocean Modifying Particles in both Hemispheres) field experiment (www.atmosphere.mpg.de/enid/oomph). The research vessel Marion Dusfresne traversed regions of relatively low marine biological productivity in the mid- and South Atlantic, and a high productivity region of a natural phytoplankton bloom in the west. The first leg was conducted from 19 January to 5 February 2007, from Cape Town, South Africa to Punta Arenas, Chile and timed to coincide with the annual phytoplankton maximum in this region. The second leg took place between 1 and 25 March 2007, from Punta Arenas, Chile to La Reunion Island, France.

Air samples

A central, high flow inlet for several trace gas instruments was installed at the top of the ship's foremast (18 m above sea level). Air was drawn rapidly (at ∼7 L min^{-1}) through a 1.27-cm outer diameter, 75 m-long shrouded Teflon line. The residence time in the line was estimated as ∼1 min.

Using the same type of cartridges as used in the laboratory, and employing the same cleaning and storage procedures, more than 100 cartridges were sampled during the first leg of the OOMPH cruise, and on the second leg a further 100 samples were taken on-line with the gas chromatograph-mass spectrometer (GC-MS) system described below. The sampling flow rate through the cartridge was 50 mL min^{-1} and the sampling duration was 1 h so that a total volume of 3 L was sampled.

Analytical procedure

A GC-MS analysis system was used to analyse the cartridges from both the laboratory and shipborne samples. This consisted of an air concentrating autosampler, and a thermal desorber (Markes Int., Pontyclun, UK), coupled to a gas chromatograph (GC6890A, Agilent Technologies, CA, USA), itself linked to a Mass Selective Detector (MSD 5973 inert) from the same company. All pertinent analysis parameters are summarised in Table 1. Laboratory multipoint calibrations of the monoterpenes and isoprene showed good linearity within the concentration ranges measured. One-point calibrations of a commercial volatile organic compound (VOC) standard (Apel-Riemer, CT, USA) were carried out at two hourly intervals. Blanks were taken regularly and showed no significant levels for the compounds discussed. The total uncertainties were between 10 and 15% in both laboratory experiments and shipboard measurements. In the laboratory 1.5 L were sampled whereas at sea 3 L were collected. The detection limits ranged from 0.5 to 5 pptv depending on the species in the laboratory study, and from 1 to 5 pptv in the marine atmospheric measurements.

Pigment samples

For phytoplankton pigment analysis, approximately every 3 h, 1–2 L of water from the ships seawater line (7 m below the ship), were filtered through GF/F filters, which were stored at −80°C until they were analysed by high performance liquid chromatography (HPLC), details are described in Hoffmann et al.[29] The taxonomic structure of the phytoplankton communities was derived from photosynthetic pigment ratios using the CHEMTAX program[30] applying the input matrix of Veldhuis and Kraay.[31] The phytoplankton group composition is expressed in chlorophyll *a* concentrations.

Back-trajectory weighted chlorophyll a exposure

For the ambient data samples, it was attempted to relate the monoterpene emissions to chlorophyll *a* concentrations in the seawater. However, simply correlating in-situ chlorophyll and observed monoterpene abundance does not account for air mass exposure to potential upwind sources or sinks from the ship sampling position. Rather than in-situ chlorophyll, we therefore used a time-series of back-trajectory weighted chlorophyll *a* to provide an estimate of the biological footprint sampled by air masses during transport to the ship. Ten-day kinematic back-trajectories initialised from the ship's position are calculated from 1.0125-degree resolution ECMWF meteorological fields using the OFFLINE trajectory model.[32] The trajectory arrival frequency is one per minute, and positions are stored every 6 h back along the trajectories. At each position along a back trajectory, concentrations of ocean chlorophyll *a* are taken from monthly mean global fields from Level-3 SeaWiFS daily binned observations provided by NASA/GSF/DAAC, degraded to 0.25-degree (∼30 km) resolution. An average chlorophyll *a* exposure was then calculated along each back trajectory, to produce a time-series of average chlorophyll *a* air mass exposure along the ship track. The use of monthly mean chlorophyll fields is a limitation, since we cannot account for variation in ocean chlorophyll on shorter timescales. In addition, the trajectories do not account for small-scale wind variability and sub-grid transport processes that may be active in the marine lower atmosphere. However, over the remote oceans, in the absence of strong convection and turbulent mixing, we expect the resolved flow in the ECMWF model to be relatively representative of the large-scale flow. The average chlorophyll values thus calculated will also have some dependence on the output frequency used for the trajectories (here 6 h). However, by degrading the resolution of the global chlorophyll fields, we have effectively spatially averaged the chlorophyll values, removing much of the bias given by the

specific overlap locations of each trajectory time point and the chlorophyll field.

Results and discussion

Emission of monoterpenes from phytoplankton

Among the nine marine phytoplankton monocultures investigated in this study, some cultures were found to have significantly enhanced levels of monoterpenes, while for others the monoterpene levels did not differ from the cultureless control. The monoterpenes were identified by combining mass spectroscopy data, elution order, and comparison of retention times of pure standards analysed under the same experimental conditions and following the analytical procedure described by Yassaa and Williams.[33] This is the first time monoterpenes have been identified as phytoplankton emissions.

Fig. 1 shows an example of the reconstructed mass chromatographic profile of monoterpenes obtained by analysing the gaseous emission of *D. tertiolecta* and the medium (overlayed in blue) on a β-cyclodextrin column using single ion mode (SIM). The chromatographic profile depicted in Fig. 1 was generated by plotting the current produced by ions with m/z 93, a fragment quite specific of monoterpene hydrocarbons that, in many cases, is the base peak of the mass spectrum. The numbers on the peaks refer to the identified compounds reported in Table 2. Nine monoterpenes were identified in the emission of *D. tertiolecta*; (−)- and (+)-α-pinene, myrcene, (+)-camphene, (−)-sabinene, (+)-δ-3-carene, (−)-β-pinene, (−)-limonene and *p*-ocimene. As reported in Table 2, *p*-ocimene accounted for 37% of the total monoterpenes and was the dominant measured monoterpene in the emission of *D. tertiolecta*. Since we have employed a β-cyclodextrin capillary separation column, the enantiomers of
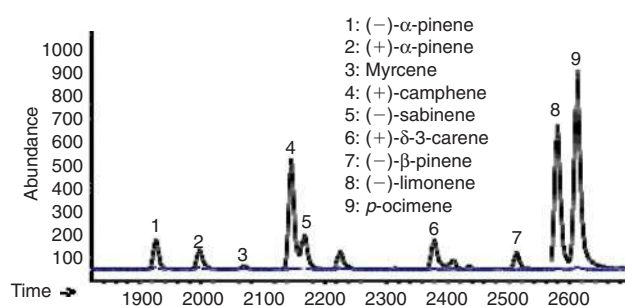
optically active monoterpenes were also well resolved. The average emission rates of the sum of monoterpenes expressed as nmol total $C_{10}H_{16}$ (monoterpene) g [chlorophyll a]$^{-1}$ day^{-1} emitted by the nine phytoplankton species are reported in Table 3. Among the phytoplankton sampled, *D. tertiolecta*, a Chlorophycae species (close to 226 nmol g [chlorophyll a]$^{-1}$ day^{-1}), was the strongest emitter of monoterpenes followed by *P. tricornutum* (∼68 nmol g [chlorophyll a]$^{-1}$ day^{-1}) and then by *F. kerguelensis* (∼43 nmol g [chlorophyll a]$^{-1}$ day^{-1}). While *D. tertiolecta* is less important in oceanic phytoplankton, *F. kerguelensis* is an important, prolific species of the Southern Ocean, whose frustules make up most of the sediment in this region.

Since terrestrial vegetation is known to emit both isoprene and monoterpenes,[34] and it has been established that marine phytoplankton do emit isoprene,[12,17] the emission of monoterpenes from phytoplankton cannot be entirely unexpected. It is worth considering that terpenes are common metabolites in marine plants and their formation by the condensation of isopentenyl pyrophosphate units is, in this regard, comparable to terrestrial metabolism.[35,36] Unlike terrestrial biosynthesis mechanisms, marine terpene production is thought to involve halogens, particularly bromine, in the primary production of terpenes through cyclisation reactions. Bromine-induced cyclisation of a slightly modified monoterpene precursor yields a bromoterpene that can then be further halogenated. This process is common in marine organisms and a variety of haloterpenoids (∼400 are known) can be produced.[35,36] There is no literature evidence of terrestrial emissions of haloterpenes, and likewise no evidence that marine phytoplankton or macroalgae produce monoterpenes with the same structures as found in terrestrial plants. However, myrcene was identified by Wise et al.[35] as a major monoterpene emission (32.6%) after (*E*)-10-bromomyrcene (33.2%) in cultured *Ochtodes secundiramea* tissue that was subjected to simultaneous steam distillation/solvent extraction and analysed by GC-MS. Previous measurements of various volatile organic compounds in ocean sediments[37] have also led the authors to speculate that marine terpenes may be precursors for biological and low temperature chemical formation mechanisms.

Fig. 1. Reconstructed mass chromatographic profiles (m/z 93) of monoterpenes present in the gaseous emission of *D. tertiolecta* and the medium (overlaid in blue).

Shipboard measurements

Following the identification of monoterpenes in monoculture samples in the laboratory we sought to directly confirm this

Table 2. Percent compositions of monoterpenes and enantiomeric compositions of chiral monoterpenes present in the gaseous emission of *D. tertiolecta*

Peak no.	R_t (min)	Monoterpenes	Percent composition	% Enantiomeric
1	19.246	(−)-α-pinene	5	57
2	19.959	(+)-α-pinene	4	43
3	20.653	Myrcene	1	
4	21.459	(+)-camphene	15	100
5	21.673	(−)-sabinene	5	100
6	23.779	(+)-δ-3-carene	5	100
7	25.113	(−)-β-pinene	3	100
8	25.788	(−)-limonene	25	100
9	26.117	*p*-ocimene	36	

Table 3. Emission rates of monoterpenes from the nine algae species. Data represent a mean of 16 measurements for each phytoplankton species

Phytoplankton species	Emission rates (nmol g [chlorophyll a]$^{-1}$ day^{-1})
Diatom	
Chaetoceros neogracilis	3.7
Chaetoceros debilis	0.8
Phaeodactylum tricornutum	68.1
Skeletonema costatum	0.3
Fragilariopsis kerguelensis	43.1
Coccolithophorids	
Emiliania huxleyi	0.3
Chlorophycae	
Dunaliella tertiolecta	225.9
Cyanobacteria	
Trichodesmium	0.8
Synechococcus	1.1

finding in ambient air. The Southern Atlantic is an ideal location to perform such measurements since there is practically no influence from advected anthropogenic pollution (in contrast to the North Atlantic) and a ship can be positioned so far from land that any short lived monoterpenes measured may be attributed to oceanic emissions.

Fig. 2 shows a reconstructed mass chromatographic profile of monoterpenes in an ambient air sample collected over the Southern Atlantic Ocean at a position of 46°99′S, 64°70′W and at maximum monoterpene levels. Monoterpenes were positively identified following the procedure described above. It can be seen that monoterpenes were detected in remote marine air, consistent with an oceanic source of monoterpenes. However, in contrast to the monoculture experiments that showed *p*-ocimene as the dominant monoterpene emission, in the South Atlantic marine boundary layer (−)- and (+)-α-pinene were the major isoprenoid compounds in all samples. *p*-Ocimene and other monoterpene species were also identified, but at much lower levels. Therefore, only (−)- and (+)-α-pinene were quantified in this study. Table 4 reports the mixing ratios range of isoprene, (−)-α-pinene, and (+)-α-pinene, the sum of (−)-α-pinene and (+)-α-pinene, and the percent composition of (−)-α-pinene and (+)-α-pinene along with in-situ chlorophyll *a* and back-trajectory weighted chlorophyll *a* during the first leg of the cruise. The cruise can be divided into three regions according to the isoprene and α-pinene mixing ratios reported in Table 4. The first region was from 35°49′S, 20°22′E to 43°18′S, 11°17′W (denoted as 'A') where these organic species were at or close to the detection limit. The other two regions showed high concentrations, the first one termed 'distant bloom (before reaching the bloom region)' from 43°37′S, 11°76′W to 41°51′S, 48°42′W (denoted as 'B', from 25 to 31 January 2007 where the maximum α-pinene level was around 115 pptv); and the second one was termed 'in-situ bloom' from 41°51′S, 48°42′W to 47°55′S, 65°47′W (denoted as 'C', from 31 January to 3 February 2007 where the maximum α-pinene level was around 225 pptv).

Fig. 3 shows that the vessel traversed regions of relatively low marine biological productivity in the mid- and South Atlantic (region A), and a high productivity region of a natural phytoplankton bloom in the west (region C). As mentioned in the previous section, in order to relate chlorophyll *a* and observed monoterpene abundance, we also used 10-day chlorophyll *a* weighted back trajectories, to provide an estimate of the biological footprint sampled by air masses during their transport to the ship. Fig. 4a, b depict the temporal variation of isoprene, (−)-α-pinene, (+)-α-pinene, and the sum of (−)- and (+)-α-pinene, along with in-situ chlorophyll *a* (calculated as the sum of divinyl-chlorophyll *a* and chlorophyll *a*) and 10-day back-trajectory weighted SeaWiFS chlorophyll *a*.

The pigment analysis showed that the areas were characterised by distinct phytoplankton compositions (Fig. 5). While in all regions, cyanobacteria built a solid background community, the low chlorophyll regions (region A) were also inhabited by a high proportion of prochlorophytes, typical for this oligotrophic region.[38] In the distant bloom area (region B), the relative proportion of green algae, namely the class of prasinophytes and chlorophytes, increased their relative distribution. *D. tertiolecta*, the profilic emitter of monoterpenes during the laboratory culture study, also belongs to the chlorophytes and thus, assuming that green algae and especially chlorophytes are strong monoterpene emitters, this might explain the increase observed in monoterpenes in this region. In the 'in-situ bloom' region (region C), the relative contribution of these groups decreases again, however, total chlorophyll has increased (Table 4), therefore, the actual concentration of this group is in the same range as before, which might explain the monoterpenes observed in region C. Since monoterpenes show the highest concentrations in this region, it is very likely that other groups in the bloom also contribute to this signal. Dinoflagellates and diatoms are dominant in some of the blooms indicated in Fig. 4a. Since dinoflagellates were not investigated in the laboratory study, we can only speculate that this group might also be
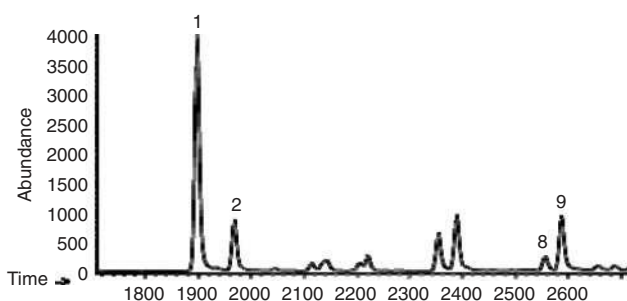

Fig. 2. Reconstructed mass chromatographic profiles (*m/z* 93) of monoterpenes present in ambient air samples collected over the Southern Atlantic Ocean at the 46°99′S, 64°70′W position.

Table 4.   Ship-board measured isoprene and α-pinene mixing ratios (pptv) in the first leg of the cruise in Southern Ocean in January–February 2007 (35°49′S, 20°22′E to 52°17′S, 67°73′W)
The data represent the results of the analysis of 60 cartridges. The far away bloom from 35°49′S, 20°22′E to 43°18′S, 11°17′W is signified by A in Figs 3 and 4; the distant bloom from 43°18′S, 11°17′W to 41°51′S, 48°42′W is signified by B; and the in-situ bloom from 41°51′S, 48°42′W to 47°54′S, 65°47′W is signified by C. u.d.l., under detection limit

| Monoterpene	Far away before the bloom			Distant bloom			In-situ bloom		
	Min.	Max.	Aver.	Min.	Max.	Aver.	Min.	Max.	Aver.
Isoprene	u.d.l.	48	26	60	138	99	32	375	187
(−)-α-pinene	u.d.l.	11	4	15	78	48	47	180	81
(+)-α-pinene	u.d.l.	2	1	10	52		10	78	44
Sum α-pinene	u.d.l.	14	5	25	130	79	56	225	125
% (−)-α-pinene	51	90	67	54	67	61	51	85	65
% (+)-α-pinene	10	49	33	33	46	39	15	49	35
In-situ chlorophyll *a* (mg m^{-3})	0.045	0.556	0.355	0.199	0.931	0.487	0.107	4.628	1.017
Averaged 10-day back-trajectories weighted chlorophyll *a* (mg m^{-3})	0.017	1.187	0.264	0.108	1.234	0.372	0.031	1.753	0.450
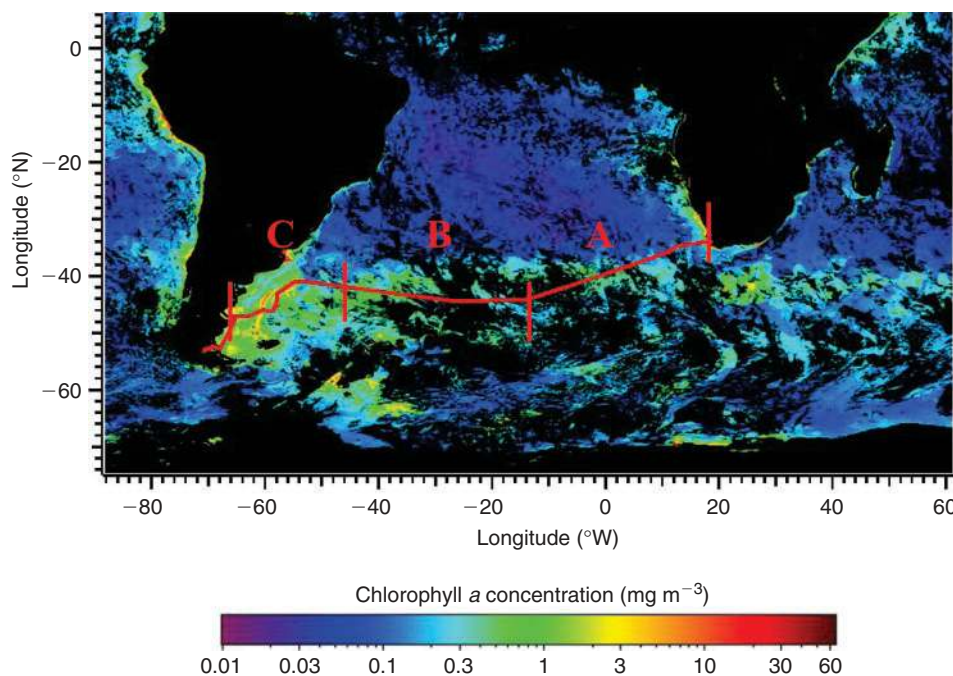
Fig. 3. A MODIS combined satellite image for January 2007 for chlorophyll. The red line indicates the research cruise track for the first leg from Cape Town (South Africa) to Punta Arenas (Chile).

a major monoterpene emitter. Another group, the pelagophytes, also not investigated during the laboratory studies, was important in region B and C and might also be responsible for some of the monoterpene emissions. Even though we are currently not able to pinpoint which group might be the most important monoterpene emitter in the field, a clear relation between the increase of phytoplankton and monoterpenes exists.

It is interesting to note that isoprene and α-pinene measured over the open ocean were correlated ($r^2 = 0.41$), in particular within the bloom region ($r^2 = 0.57$), which suggests that these two species have a similar emission source. In general, both isoprene and α-pinene appear to correlate with the 10-day averaged back-trajectory weighted chlorophyll a (see Fig. 4b), especially under the influence of the main phytoplankton 'in-situ bloom', again consistent with an oceanic phytoplankton-type source. It should be noted that a strong correlation between the simulated chlorophyll a values and the monoterpenes cannot be expected since phytoplankton speciation, chlorophyll content and well being (physiological state) vary greatly, and are likely to give rise to diverse monoterpene emission strengths. Moreover, the aforementioned laboratory study has highlighted the large variation in emission rates as a function of species. Furthermore, the weighted trajectories are necessarily based on monthly average chlorophyll distributions, which may differ slightly from the actual oceanic chlorophyll content encountered by the trajectories at specific times. From Fig. 4a, b and Table 4, it can be seen that with the 10-day averaged back-trajectory weighted chlorophyll a, the obtained correlation is better than with in-situ measured chlorophyll a. Closer inspection of Fig. 4b shows that the trajectories must have crossed a higher chlorophyll region of a 'distant bloom' some days before reaching the ship. This can be seen as two peaks that appear in the trajectory trace (the first one on 26 January around 1900 hours (UTC) and the second one on 27 January around 2200 hours (UTC)). The distant bloom region responsible for these peaks must have

been emitting both isoprene and terpenes. Since the isoprene and monoterpene levels from the 'distant bloom' (region B) are only approximately two times smaller than those seen from the 'in-situ' bloom (region C) that we subsequently traversed, and come from a more distant region, we can argue, assuming similar wind speeds, that the more distant high chlorophyll region must have been a stronger emitter than the in-situ bloom. This is because some isoprene/monoterpenes will have been lost during the 3–4 days transport time as a result of oxidation by OH and O$_3$.

Since monoterpenes, and α-pinene in particular, are known to originate from terrestrial plants, we performed 10-day back-trajectories for the entire cruise track to determine the extent of land influence on the air samples. Figs 6 and 7 show the back-trajectories for the two regions of elevated monoterpene mixing ratios, respectively, while Fig. 8 represents the back-trajectories in a region where α-pinene was close to the detection limit. Both Figs 6 and 7 show that the sampled air masses were not influenced by continental influence in the past 10 days. Thus for land plants to have been responsible for the ∼50 pptv monoterpenes observed over the ocean in the section highlighted in Fig. 6, the mixing ratios at source (over 10 days away) would have to have been in excess of 1 ppm, which is several orders of magnitude higher than any previously reported terrestrial measurements. This assumes an OH level of 5×10^5 molecules cm^{-3} over the 10-day trajectory for α-pinene. We, therefore, conclude that the measured monoterpenes were most likely derived from ocean emissions.

Fig. 6 shows that the air masses in region B come predominantly from Antarctica. The high monoterpene and isoprene mixing ratios observed in the distant bloom region (zone 'B' in Fig. 4) may originate from diatoms living in sea ice in Antarctica or be emitted from regions between Antarctica and the ship. In any case we can exclude a terrestrial plant source. The back-trajectories displayed in Fig. 7 show that air masses traversing the
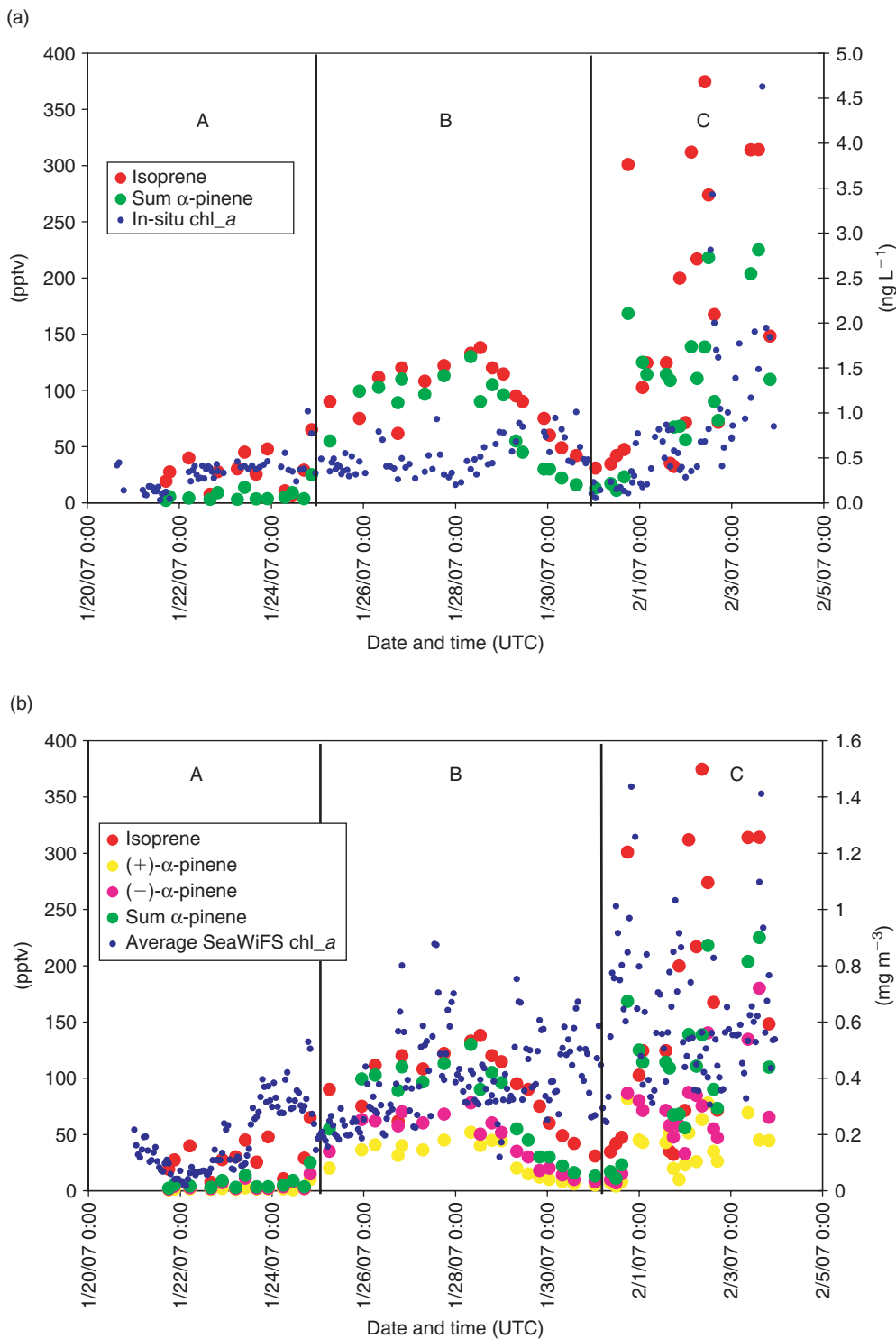
(a)

(b)

Fig. 4. Temporal variation of isoprene and α-pinene with in-situ measured chlorophyll *a* (a) and 10 days of back-trajectory weighted chlorophyll *a* (b).

main bloom (high chlorophyll region) originated from Antarctica and the Southern Pacific Ocean but passed over the southern part of South America before reaching the ship. In this case we cannot exclude an influence of terrestrial vegetation. Fig. 8 shows that although some back trajectories travelled near land, the α-pinene mixing ratios were still very low and close to the detection limit. This implies that either they were not influenced despite their proximity to land, or any terpenes emitted from this region have

oxidised before reaching the ship. This presents further evidence that the monoterpenes observed over the open ocean were produced in-situ by marine phytoplankton and not influenced by terrestrial vegetation.

From the results of laboratory experiments and shipboard measurements we deduce that a suite of monoterpenes (including those identified in this work) are emitted by phytoplankton into the surrounding waters, and because of their low Henry Law
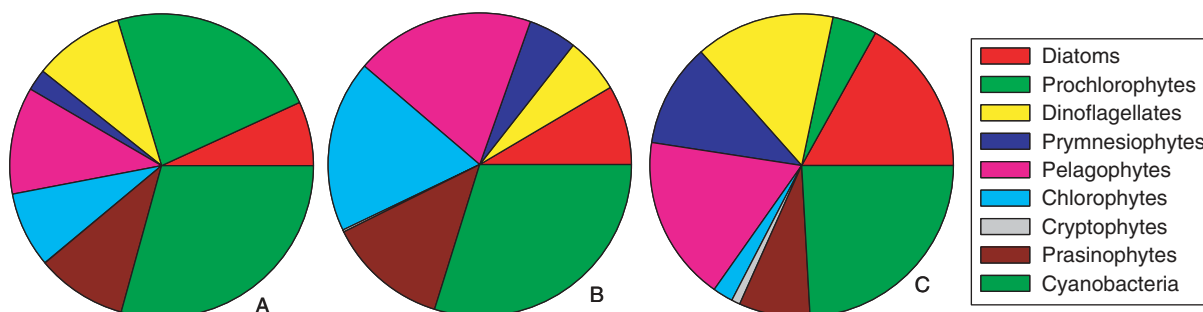
Fig. 5. Average pigment-based phytoplankton composition calculated with the CHEMTAX program for the three regions indicated in Fig. 3.
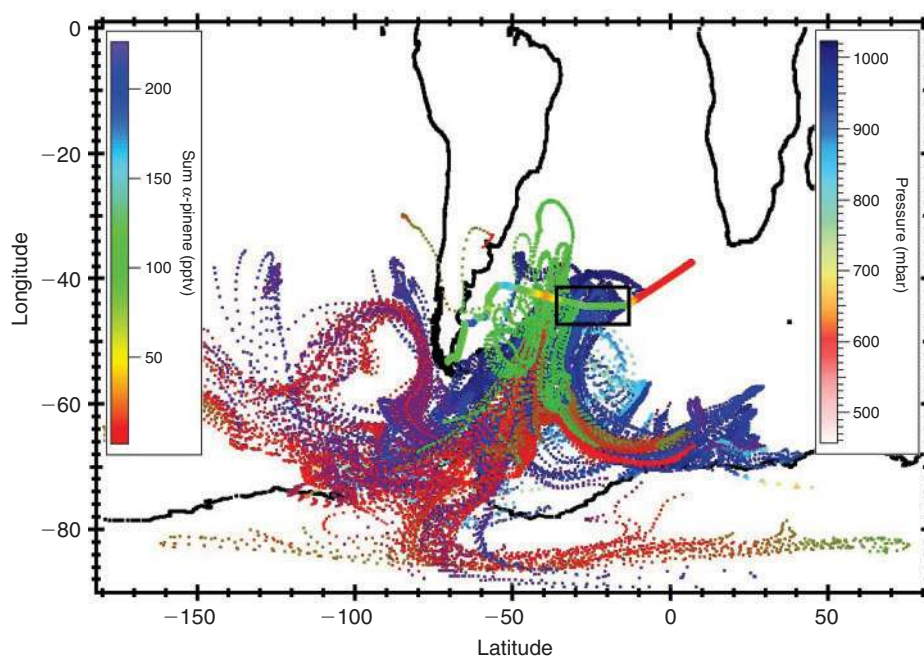

Fig. 6. Back-trajectories for the first maximum α-pinene mixing ratios (from 43°18′S, 11°17′W to 41°51′S, 48°42′W) in the region distant from the highest chlorophyll concentration termed 'distant bloom' (noted 'B' in Fig. 4). The black box marks the section of the track from where trajectories are started. The colour scale on the ship track gives the α-pinene mixing ratios while the colour scale on the back trajectories indicates the pressure altitude.

coefficients, they are then emitted into the marine boundary layer air. It is not known whether such emissions simply leak through algal tissues or whether they are emitted as a response to biological (e.g. defence against predation) or mechanical (temperature, injury) stress.

Conclusions

Laboratory experiments and shipboard measurements over the Southern Atlantic Ocean have allowed the identification of marine monoterpene emissions for the first time. The phytoplankton emission rates of total monoterpenes were low and varied from 0.3 nmol g [chlorophyll a]$^{-1}$ day^{-1} for *S. costatum* and *E. huxleyi* to 225.9 nmol g [chlorophyll a]$^{-1}$ day^{-1} for *D. tertiolecta*. The maximum atmospheric monoterpene mixing ratios of 100–225 pptv were observed in the marine atmosphere of the Southern Atlantic Ocean in the region of one phytoplankton bloom where back trajectories show no land influence for the past 10 days.

These first results highlight the need for further studies on algae species in laboratory experiments and shipboard measurements to determine the levels and atmospheric impact of monoterpenes in other areas of the marine boundary layer. Even though the ambient air concentrations of monoterpenes are low, they may, because of their high reactivity, influence the photochemistry of the remote marine atmosphere. Small quantities of marine photochemically produced isoprene (a few pptv) have been shown to impact the formaldehyde budget at a remote coastal site in the southern hemisphere.[39] This is despite the estimated global marine source of isoprene (0.1 Tg year^{-1})[40] being dwarfed by the terrestrial source (~500 Tg year^{-1}). Monoterpenes also form formaldehyde efficiently by photooxidation. However, the low levels of isoprene and monoterpenes measured here, namely over an order of magnitude less than terrestrial forested regions (see e.g. ref. [41]), indicate that the impact of marine monoterpene emissions on the formaldehyde budget will be small indeed. Nonetheless, in regions of high biological activity, e.g. the Southern Atlantic
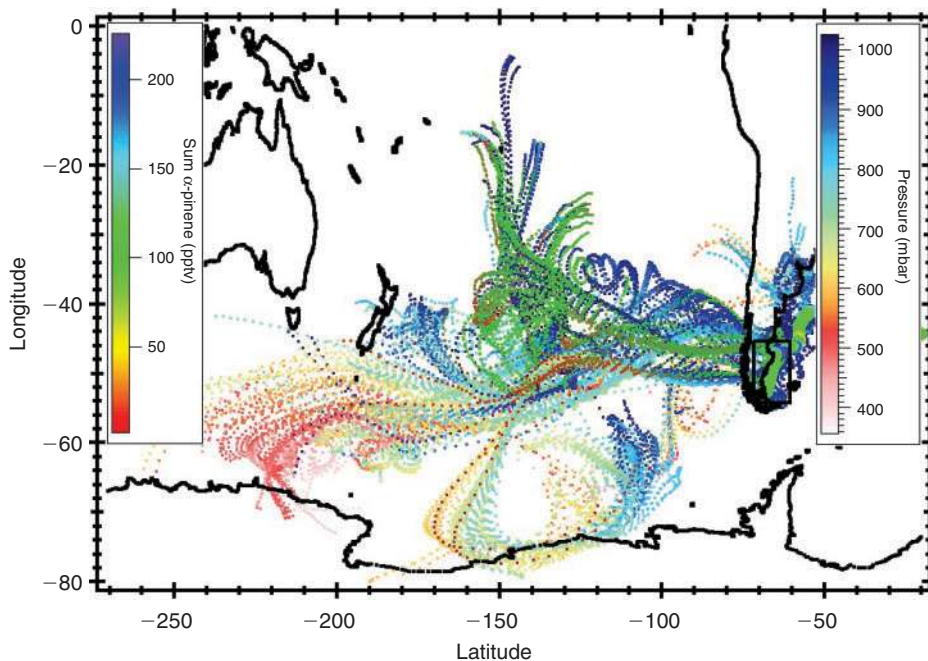
Fig. 7. Back-trajectories for the second maximum α-pinene mixing ratios (from 41°51′S, 48°42′W to 47°54′S, 65°47′W) in the region that corresponds to high in-situ chlorophyll termed 'in-situ bloom' (noted 'C' in Fig. 4). The black box marks the section of the track from where trajectories are started. The colour scale on the ship track gives the α-pinene mixing ratios while the colour scale on the back trajectories indicates the pressure altitude.
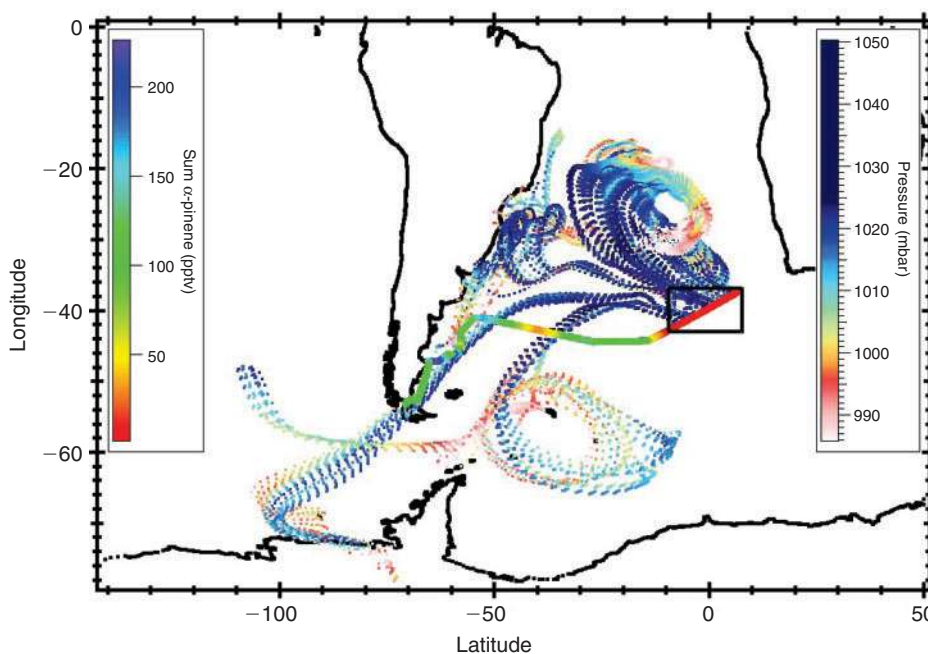
Fig. 8. Back-trajectories for the low α-pinene mixing ratios in a region far away before the bloom (noted 'A' in Fig. 4). The black box marks the section of the track from where trajectories are started. The colour scale on the ship track gives the α-pinene mixing ratios while the colour scale on the back trajectories indicates the pressure altitude.

Ocean during the bloom period, such emissions could be of importance to local photochemistry. A further consideration is that the atmospheric oxidation products of these marine emissions, albeit minor in the absolute sense, may condense on existing aerosol and thereby change its physical properties such as reflectivity and hydroscopicity. Further investigations are needed to determine the potential effect of such higher molecular weight emissions from the ocean. Such studies should also determine possible driving factors for emission, such as ambient light, CO_2 levels, temperature, growth stage, nutrient availability,

and physiological and ecological state. Moreover, in future, marine ecosystem communities should also be investigated since zooplankton and bacteria may also influence emissions.

Acknowledgements

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